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# New Actinomycetes of Commercial Importance

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## INTRODUCTION

Actinomycetes have long been exploited commercially as a rich source of novel secondary metabolites, such as antibiotics, enzymes, and enzyme inhibitors. New selective isolation techniques are continually being developed to provide new and unique actinomycete strains from nature to be screened for the production of marketable metabolites (1). Actinomycete strains isolated using particularly novel approaches or from unusual sources may be members of hitherto undescribed genera or species. Advances in actinomycete systematics have resulted in much better description of new taxa, as well as the reclassification of existing strains into new genera (5, 16), through the application of chemotaxonomic and molecular genetic criteria. Potential commercially important taxa resulting from both of these activities will be discussed in this paper.

## DISCUSSION

In the course of isolating actinomycete strains from soil samples and screening them for novel antibiotic production potential, two new genera were discovered.

**The genus *Saccharothrix* Labeda et al, 1984.** The first strain recognized as a member of this genus, NRRL 11239, was isolated from a soil sample collected in Australia, and was of interest because it produced the broad spectrum antibiotic MB782 alpha (Tresner et al, U.S. Patent 4,234,717, November 18, 1980). This strain was originally identified as a *Nocardia* species based on the fragmentation of both the substrate and aerial mycelium into square ended elements. A careful analysis of the cell wall composition of this strain revealed that, although it contained the *meso*-isomer of diaminopimelic acid (DAP), the whole-cell sugar pattern consisted of rhamnose and galactose.

This is not a typical type IV cell wall *sensu* Lechevalier et al (13), since arabinose is lacking in the whole-cell sugar pattern and, moreover, its cells do not contain nocardomycolic acids found in cells of *Nocardia* species. Thus, it could not be a member of this genus. Strains of *Saccharothrix* are morphologically very similar to strains of the genus *Nocardiopsis*, but the members of this latter genus do not have rhamnose present in whole cell hydrolysates as a diagnostic sugar. This strain was found to contain phosphatidylethanolamine as the diagnostic nitrogenous phospholipid, along with diphosphatidyl glycerol, phosphatidylinositol, and phosphatidylinositol mannosides, thus making it a type PII phospholipid pattern (12). The major menaquinones present are of the MK-9 series and appear to be tetraydrogenated (9). The chemotaxonomic properties of this genus as compared to other spore-forming genera are shown in Table 1. The Australian soil isolate was designated as the type species and was named *Saccharothrix australiensis* (9). The type strain of "*Nocardia aerocolonigenes*" was also found to chemotaxonomically fit the genus *Saccharothrix* and was transferred as *Saccharothrix aerocolonigenes* (7). The physiological differences between *S. australiensis* and *S. aerocolonigenes* are shown in Table 2.

Apparently, *Saccharothrix* strains may be selectively isolated with media containing 5 to 10 micrograms/ml of penicillin G and 15 micrograms/ml of nalidixic acid (M. Shearer, personal communication). The genus appears to be fairly ubiquitous in soils, and several other isolates, as yet undescribed, have been obtained from other soil samples. Recently, a U.S. Patent was issued for a process in which a strain of *Saccharothrix (Nocardia) aerocolonigenes* is used to produce the antibiotic rebeccamycin (Nettleton et al. U. S. patent 4,552,842, November 12, 1985). The commercially important metabolites produced by *Saccharothrix* strains are shown in Table 3. Some strains otherwise fitting into the genus *Saccharothrix* morphologically and chemotaxonomically

Table 1. A comparison of the chemotaxonomic profiles of actinomycete genera that form aerial spore chains

| Genus | Cell Wall Type | Whole Cell Sugars | Phospholipid Group | Principle Menaquinones | DNA Mol% G+C |
|---|---|---|---|---|---|
| *Actinomadura* | III | Madurose | PI | MK-9(H4), MK-9(H6) | 66-70 |
| *Actinopolyspora* | IV | Arabinose, Galactose | PIII | MK-9(H4) | 64 |
| *Amycolata* | IV | Arabinose, Galactose | PIII | MK-8(H2) MK-8(H4) | 68-72 |
| *Amycolatopsis* | IV | Arabinose, Galactose | PII | MK-9(H2) MK-9(H4) | 66-69 |
| *Glycomyces* | II | Xylose, Arabinose | PI | MK-10(H2), MK-10(H6) | 71-73 |
| *Microtetraspora* | III | Madurose | PI, PIV | MK-9(H4) | NA |
| *Nocardia* | IV | Arabinose, Galactose | PII | MK-8(H4), MK-9(H2) | 64-72 |
| *Nocardioides* | I | None | PI | MK-8(H4) | 66-67 |
| *Nocardiopsis* | III | None | PIII | MK-10(H4), MK-10(H6) | 64-69 |
| *Pseudonocardia* | IV | Arabinose, Galactose | PIII | MK-9(H4) | 79 |
| *Saccharopolyspora* | IV | Arabinose, Galactose | PIII | MK-9(H4) | 77 |
| *Saccharothrix* | III | Rhamnose, Galactose | PII | MK-9(H4) | 70-76 |
| *Streptomyces* | I | None | PII | MK-9(H6), MK-9(H8) | 69-78 |

Table 2. Differential physiological characteristics of *Saccharothrix* species

| | *S. australiensis* | *S. aerocolonigenes* |
|---|---|---|
| Decomposition of: | | |
| Hypoxanthine | – | + |
| Potato starch | – | + |
| Urea | – | Variable |
| Acid from: | | |
| Arabinose | – | + |
| Erythritol | + | – |
| Inositol | – | + |
| Lactose | – | + |
| Melibiose | – | + |
| Raffinose | – | + |
| Rhamnose | – | + |
| Salicin | – | + |
| Sorbitol | + | – |
| Sucrose | – | + |
| Xylose | – | + |
| Utilization of: | | |
| Citrate | – | + |
| Lactate | Variable | + |
| Oxalate | – | + |
| Tartrate | – | + |
| Production of: | | |
| Phosphatase | – | + |
| Growth at: | | |
| 42°C | + | Variable |
| 45°C | + | – |

have been found to have a Type PIV phospholipid pattern (glucosamine-containing phospholipids also present) rather than the Type PII phospholipid pattern described for the genus (9), and the genus description may need to be amended to accommodate these strains.

**The genus *Glycomyces* Labeda et al, 1985.** The original strain of this new genus, NRRL 15337, was isolated from a soil sample from Harbin, People's Republic of China, in 1981. This strain produces both azaserine and a derivative of azaserine, LL-D05139 beta. Strains of this genus produce chains of spores on aerial sporophores, but the substrate mycelium is not subject to fragmentation. The cell walls contain the *meso*-isomer of diaminopimelic acid and D-glycine (along with L-alanine, D-alanine, D-glutamic acid, glucosamine and muramic acid), and the

Table 3. Commercially important secondary metabolites from *Glycomyces* and *Saccharothrix* species

| Species | Strain | Product |
|---|---|---|
| *Glycomyces harbinensis* | NRRL 15337 | Azaserine, DO5139 beta |
| *Saccharothrix australiensis* | NRRL 11239 | Antibiotic BM-782 |
| *Saccharothrix aerocolonigenes* | ATCC 39243 | Rebeccamycin |

whole-cell sugar pattern consists of xylose and arabinose (sometimes in minute quantities), corresponding to a Type II cell wall and whole-cell sugar pattern D *sensu* Lechevalier and Lechevalier (13). No nitrogenous phospholipids are observed in extracts of *Glycomyces* strains, thus categorizing them as having a type PI phospholipid pattern (12). Although this phospholipid pattern and morphology are also observed for the genera *Actinomadura* and *Nocardiopsis*, the cell wall composition of these genera is clearly different from that of *Glycomyces* (see Table 1). The cell wall composition of this genus is more typical of members of the *Actinoplanaceae* and *Micromonosporaceae* but they exhibit a totally different sporulation micromorphology, i.e., production of spores in sporangia in the former family versus production of single spores born in the substrate mycelium in the latter family. The genus *Glycomyces* was created to accommodate strains having these morphological and chemotaxonomic properties, and *G. harbinensis* was named as the type species (10). Another isolate from a greenhouse soil from New Jersey proved to be physiologically different from *G. harbinensis* and only exhibited 22 to 30% DNA relatedness to the type species. It was thus designated as a new species and was named *G. rutgersensis* (10). A comparison of the physiological properties of these species is shown in Table 4.

Both of these strains are resistant to 25 micrograms/ml of novobiocin and 10 micrograms/ml of streptomycin, so that a combination of these two antibiotics might be used to selectively isolate additional members of this genus. Very few additional strains of this genus have been isolated, so it appears to be rather rare in soils.

**Table 4.** Differential physiological characteristics of *Glycomyces* species

| | *G. harbinensis* | *G. rutgersensis* |
|---|---|---|
| Decomposition of: | | |
| Gelatin | – | Weak |
| Acid from: | | |
| Adonitol | – | + |
| Lactose | + | Weak |
| Melezitose | – | Weak |
| Utilization of: | | |
| Citrate | + | – |
| Lactate | – | + |
| Succinate | + | – |
| Production of: | | |
| Nitrate reductase | Weak | + |
| Growth in presence of: | | |
| 5.0% NaCl | – | Weak |
| Growth at: | | |
| 42°C | – | + |

**Genera *Amycolata* and *Amycolatopsis* Lechevalier et al 1986.** The genus *Nocardia* was one of the largest and most medically and commercially important genera in the *Actinomycetales*. Since strains were primarily assigned to this genus on the basic of morphology alone prior to the advent of chemotaxonomic criteria for classification, it is not surprising that the majority of the strains originally assigned to this genus have been subsequently moved to other genera. As presently defined, the genus *Nocardia* is comprised of nocardioform (i.e., vegetative hyphae tend to fragment into small, squarish units) organisms that have a Type IV cell wall composition (*meso*-DAP, and arabinose and galactose present), type PII phospholipids (phosphatidyl ethanolamine and phosphatidyl methylethanolamine present as nitrogenous phospholipids), and contain nocardomycolic acids. It was found that a number of "*Nocardia*" species lacked mycolic acids, although they have a cell wall composition and micromorphology similar to the true *Nocardia* (3, 5, 14). Two new genera, *Amycolata* and *Amycolatopsis*, were proposed to accommodate some of these strains (16). These two new genera can be distinguished from each other based on phospholipid and menaquinone composition, as can be seen in Table 1. *Amycolata* strains have a type PIII phospholipid pattern (phosphatidylcholine present as nitrogenous phospholipid) and have menaquinones that predominantly have eight isoprenoid units in the side chain (MK-8 series). *Amycolatopsis* strains have a type PII phospholipid pattern (phosphatidylethanolamine and phosphatidylmethylethanolamine as nitrogenous phospholipids) and menaquinones that predominantly have nine isoprenoid units in the side chain (MK-9). Moreover, *Amycolata* species are not susceptible to lysis by *Amycolatopsis* phage (16). The species of the genus *Amycolatopsis* and the commercially important secondary metabolites produced by them are shown in Table 5. The differential physiological properties of these species are shown in Table 6. The described species of the genus *Amycolata* are shown in Table 7. Two of these species, *A. autotrophica* and *A. saturnea* are reported to grow autotrophically, and the remaining species, *A. hydrocarbonoxydans*, utilizes hydrocarbons for growth.

***Saccharopolyspora erythraea*, sp. nov.** The erythromycin-producing strains of *Streptomyces erythraeus* are of great comercial importance because this antibiotic is still widely used in medicine. Kuznetzov et al (6) reported that the cell walls of the type strain of *S. erythraeus*

Table 5. Commercially important secondary metabolites of *Amycolatopsis species*

| Species | Strain | Product | Reference |
|---|---|---|---|
| *Amycolatopsis mediterranei* | NRRL B-3240 | Rifamycins | (17) |
| *Amycolatopsis orientalis* | NRRL 2450 | Vancomycins | (18) |
| *Amycolatopsis orientalis* subsp. *lurida* | NRRL 2430 | Ristocetin | (4) |
| *Amycolatopsis rugosa* | NRRL 2295 | Vitamin B12 | (2) |
| *Amycolatopsis sulphurea* | NRRL 2822 | Chelocardin | |

contained the *meso*-isomer of DAP and arabinose and galactose, clearly a cell wall Type IV, not characteristic of members of the genus *Streptomyces*. They suggested that this species be placed in the genus *Proactinomyces*, but subsequent studies in our laboratory showed that cells of *S. erythraeus* NRRL 2338, the type strain, lacked nocardomycolic acids. This strain has a type PIII phospholipid pattern (phosphatidyl choline present as nitrogenous phospholipid) (12), and the principal menaquinones present are MK-9 (H2) and MK-10 (H2). The chemotaxonomic profile observed for this strain precluded its inclusion into either *Amycolata* or *Amycolatopsis*, but matched those reported for the genus *Saccharopolyspora*. The morphology of *S. erythraeus* also showed that it, readily fits into this genus, so it was proposed that the erythromycin-producing strains be transferred to the genus *Saccharopolyspora* as *Saccharopolyspora erythraea* (8). The DNA isolated from *S. erythraea* NRRL 2338 was found to be 24% homologous to that from *S. hirsuta* NRRL B-5792, the type strain of this species, illustrating that they represent different species, but actinophage isolated on *S. erythraea* have been found to lyse *S. hirsuta* strains (C. Hutchinson, personal communication; R. Stanzak, personal communication) confirming that they are proba-

Table 6. Differential physiological characteristics of *Amycolata* species

| | *Amycolata autotrophica* | *Amycolata hydrocarbo-noxydans* | *Amycolata saturnea* |
|---|---|---|---|
| Decomposition of: | | | |
| Adenine | + | – | – |
| Hypoxanthine | – | – | + |
| Tyrosine | – | – | + |
| Xanthine | – | – | + |
| Decarboxylation of | | | |
| Benzoate | – | – | + |
| Citrate | + | – | – |
| Degradation of: | | | |
| Gelatin | – | + | + |
| Starch | – | + | – |
| Urea | + | – | + |
| Growth in: | | | |
| 5.0% NaCl | + | – | – |
| Acid produced from: | | | |
| Adonitol | + | – | – |
| Erythritol | + | + | – |
| Galactose | + | + | – |
| Lactose | – | + | – |
| Maltose | + | – | + |
| Mannitol | + | – | + |
| alpha–Methyl–D–glucoside | + | – | + |
| Rhamnose | – | + | – |
| Salicin | – | + | – |
| Sorbitol | + | – | – |
| Trehalose | + | – | + |

Table 8. Differential physiological characteristics of *Saccharopolyspora* sp.

| | *Saccharopolyspora hirsuta* NRRL B-5792 | *Saccharopolyspora erythraea* NRRL 2338 |
|---|---|---|
| Decomposition of: | | |
| Casein | + | V[a] |
| Xanthine | + | W[b] |
| Decarboxylation of: | | |
| Benzoate | + | – |
| Mucate | + | – |
| Oxalate | V | – |
| Propionate | + | V |
| Tartrate | + | – |
| Production of: | | |
| Nitrate reductase | – | + |
| Growth in: | | |
| Salicylate | V | – |
| Lysozyme broth | – | W |
| Growth at: | | |
| 45°C | + | – |
| 50°C | V | – |
| Acid produced from: | | |
| Arabinose | – | + |
| Erythritol | – | + |
| Lactose | + | – |
| Melibiose | – | + |
| Melezitose | + | – |
| alpha-methyl-D-glucoside | + | – |
| Raffinose | V | + |
| Sorbitol | + | V |

[a] V = Variable reaction.
[b] W = Weak positive reaction.

**Table 7.** Differential physiological characteristics of *Amycolatopsis* species

| | *Amycolatopsis orientalis* | *Amycolatopsis orientalis subspecies lurida* | *Amycolatopsis mediterranei* | *Amycolatopsis rugosa* | *Amycolatopsis sulphurea* |
|---|---|---|---|---|---|
| Decomposition of: | | | | | |
| Xanthine | + | + | – | + | – |
| Decarboxylation of: | | | | | |
| Benzoate | – | – | – | + | – |
| Citrate | + | + | + | – | + |
| Production of: | | | | | |
| Nitrate reductase | + | + | + | – | + |
| Phosphatase | + | – | + | + | + |
| Degradation of: | | | | | |
| Urea | + | + | + | + | – |
| Growth in or on: | | | | | |
| Lysozyme broth | – | Weak | + | – | + |
| Salicylate | – | – | + | – | – |
| 5% NaCl | + | Variable | – | + | + |
| Growth at: | | | | | |
| 10°C | + | + | + | + | – |
| 45°C | – | – | – | + | – |
| Acid from: | | | | | |
| Adonitol | + | + | – | + | – |
| Arabinose | + | + | + | + | – |
| Cellobiose | + | + | + | – | – |
| Dextrin | + | + | + | – | + |
| Erythritol | + | + | – | + | – |
| Inositol | + | + | + | – | + |
| Lactose | + | + | + | – | – |
| Maltose | + | + | + | – | + |
| Melibiose | + | – | + | – | – |
| alpha-Methyl-D-glucoside | + | + | + | – | – |
| Raffinose | – | – | + | – | – |
| Rhamnose | + | – | + | + | – |
| Salicin | + | + | + | + | – |
| Sucrose | + | + | + | – | + |
| Xylose | + | + | + | + | – |

bly related at the genus level. The differential physiological characteristics of *S. erythraea* compared with *S. hirsuta* are shown in Table 8.

The development of new media and techniques for the isolation of unique actinomycetes from the natural environment will continue to provide sources of novel secondary metabolites for commercial exploitation. As the latest chemotaxonomic and molecular taxonomic criteria are applied to older described taxa it is likely that a number of them are misclassified and will more correctly belong to more recently described genera.

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